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7044

FUMONISINS, MYCOTOXINS PRODUCED BY *FUSARIUM* SPECIES: BIOLOGY, CHEMISTRY, AND SIGNIFICANCE [1]

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INTRODUCTION

Mycotoxins are secondary metabolites of fungi that are harmful to both animals and humans. Mycotoxicoses are diseases caused by the ingestion of foods or feeds made toxic by these fungal metabolites. Mycotoxins undoubtedly have presented a hazard to human and animal health for decades, and this threat only can become more important as the demand on the available food supply increases with the increase in population. If the food supply is limited, the mycotoxin hazard increases since more fungus-damaged, potentially mycotoxin-containing foodstuffs are consumed rather than discarded,

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and malnutrition enhances the susceptibility to lower concentrations of food-borne mycotoxins.

Mycotoxicoses are diseases in which the interaction of multiple factors in the causal complex must be considered. Some of the factors involved in the occurrence of a field outbreak of a mycotoxicosis caused by a plant pathogenic fungus are (*a*) the infection of a susceptible host plant by a mycotoxin-producing fungus; (*b*) environmental and other factors favorable to the development of the disease; (*c*) genetic capability of the pathogen to produce a metabolite or metabolites harmful to animals or humans; (*d*) environmental and other conditions favorable to the production and the accumulation of sufficient quantities of toxic metabolites in the diseased plant to cause toxicosis in the consumer, and (*e*) the consumption of sufficient quantities of toxin-containing plant material by a susceptible consumer (26).

Many species of *Fusarium* produce a number of secondary metabolites that cause different physiological and pharmacological responses in plants and animals. *Fusarium* species are best known for the production of the trichothecene mycotoxins, but they may also produce a variety of other compounds such as other mycotoxins, pigments, antibiotics, and phytotoxins (53). Since the range of compounds produced and the literature dealing with these compounds is so extensive (27), we have limited this review to a discussion of *F. moniliforme* J. Sheld. and a group of toxins, the fumonisins, discovered in 1988 (2), that are produced by this and related species of *Fusarium*.

Fusarium moniliforme, one of the most prevalent fungi associated with human and animal dietary staples such as corn (27, 33), has been suspected of being involved in human and animal diseases since its original description in 1904 (46). In the early 1900s, widespread field outbreaks of animal diseases occurred in the United States associated with the ingestion of moldy corn (39). Peters (39) reported that the hooves of cattle and horses were sloughed, pigs shed their bristles, chickens lost their feathers, some animals developed convulsions, and high percentages of affected animals died. *Fusarium moniliforme* was the fungus most commonly associated with moldy corn and was implicated as the cause of the disease "moldy corn toxicosis," described by Peters (39). More recently in some areas of the world *F. moniliforme* has been associated with high rates of human esophageal cancer. In southern Africa, the highest human esophageal cancer rate occurs in the southwestern districts of the Transkei where corn is the main dietary staple (30). The cultures of several isolates of *F. moniliforme* from corn produced in these districts were acutely toxic to ducklings (21). When culture material of these isolates grown on autoclaved corn was fed to experimental animals, the lesions induced included cirrhosis and nodular hyperplasia of the liver and intraventricular cardiac thrombosis in rats, leukoencephalomalacia and toxic hepatosis in horses, pulmonary edema in pigs, nephrosis and hepatosis in sheep, and acute congestive heart failure in baboons (20, 21). Corn-based feed naturally contaminated with *F. moniliforme* and associated with outbreaks of equine leukoencephalomalacia was fed to rats. This feed caused changes in gross liver morphology that consisted of multiple hepatic nodules and pale depressed hepatic areas. Histological examination also revealed multiple hepatic neoplastic nodules and large areas of adenofibrosis as well as cholangiocarcinomas (56). Marasas et al (25) found that isolate MRC 826 of *F. moniliforme* grown on autoclaved corn was hepatocarcinogenic to rats.

Historical Perspective of Mycotoxins Produced by Fusarium moniliforme

Toxic cultures of *F. moniliforme* and closely related species have long been known. However, this species has never been reported to produce trichothecenes, the best known and most potent mycotoxins produced by many species of *Fusarium*. Many other secondary metabolites, some of which show phytotoxicity, including various antibiotics, quinoid and napthazarine pigments, fusaric acid and gibberellins (53), have been isolated from cultures of *F. moniliforme*. Moniliformin, an acute avian mycotoxin, and fusarins, a family of modified carotenoids that are mutagenic in the Ames test, have received particular attention.

MONILIFORMIN Moniliformin was first reported to be produced by an isolate of *F. moniliforme* from corn kernels in the United States (7). This isolate lost the ability to produce moniliformin during the course of a study to determine the structure of the toxin (47). Moniliformin was eventually isolated and chemically characterized from a high-producing strain of *F. moniliforme* isolated from millet in Nigeria (47). However, this strain from millet was recently identified as *F. nygamai* because it produces chlamydospores (28). There are conflicting reports in the literature regarding the production of moniliformin by cultures of *F. moniliforme*. A study of moniliformin production in *Fusarium* section *Liseola* (29) showed that of 58 strains of *F. moniliforme* from Africa that were toxic to ducklings only 13 produced moniliformin in culture on autoclaved corn. The other 45 strains isolated from corn in Kenya, South Africa, and the Transkei were toxic to ducklings but did not produce chemically detectable amounts of moniliformin in culture. Overall, only 22% of the toxic strains produced chemically detectable amounts of moniliformin and the mean yield was low (29). In particular, of strains of *F. moniliforme* from corn, only six strains produced moniliformin and these produced small amounts. Cultures that induced leukoencephalomalacia in equine animals did not produce moniliformin. Thus, it appears that this species generally is a poor producer of moniliformin, that many toxic strains, in particular those isolated from corn, do not produce moniliformin, and that other species of *Fusarium*, such as *F. subglutinans*, are better producers of moniliformin (29).

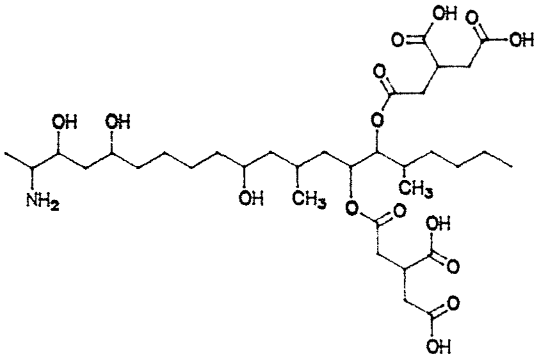
FUSARINS Mutagenicity assays of isolates of *F. moniliforme* grown on autoclaved cracked corn showed that extracts of 21 of 33 isolates were active against strain TA100 from *Salmonella typhimurium*(3). Most (70%) of the isolates from corn and an isolate from sorghum were mutagen producers (3). In 1983, Gelderblom et al (12) showed that strains of *F. moniliforme* isolated from Transkeian corn produced compounds that were mutagenic in the *Salmonella* assay. The *Salmonella* assay was subsequently used as a monitoring system for isolation of mutagenic compounds from cultures of strain MRC 826 of *F. moniliforme*. The main mutagenic compound purified from this strain of *F. moniliforme* was identical to fusarin C independently isolated from a culture of the same species by Wiebe & Bjeldanes (55). Because fusarin C was a mutagenic metabolite of *F. moniliforme*, experiments were carried out to determine the cancer-initiating activity of fusarin C. However, results obtained (11) did not provide any data to support the theory that fusarin C was carcinogenic, and they did not indicate a positive role for this compound in the hepatocarcinogenicity of strain MRC 826 of *F. moniliforme*.

Discovery of Fumonisins

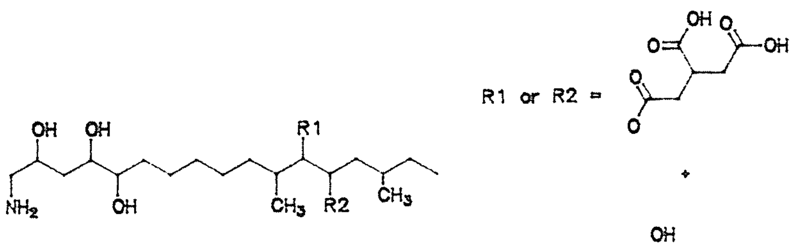
The structures of the fumonisins were first reported by a group of South African researchers in 1988 (2, 9). The discovery of fumonisins came as a result of a decade of search by that group for a possible cause of the high incidence of esophageal cancer in the Transkei region of southern Africa. Isolation of fumonisin B_1 was guided by a bioassay based on short-term cancer initiation-promotion in rat liver. The most active component in fractionated corn cultures of *F. moniliforme* MRC 826 was designated fumonisin B_1 and was shown to be a diester of propane-1,2,3-tricarboxylic acid and a 2-amino-12,16-dimethyl, 3,5,10,14,15-pentahydroxyicosane with both C-14 and C-15 hydroxy groups esterified with the terminal carboxy group of the acids (Figure 1). Fumonisin B_1 was also independently isolated and characterized by a research group from New Caledonia (22).

In addition to fumonisin B_1, several related fumonisins have been reported. Fumonisin B_2 (2) and fumonisin B_3 (6, 42) are homologs that lack one of the free hydroxyl groups on the backbone. Fumonisin B_2 lacks the hydroxyl at C-10 while fumonisin B_3 lacks the hydroxyl at C-5. These three fumonisins account for most of the fumonisins that occur in naturally contaminated corn samples.

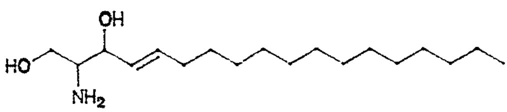
Some samples of corn contain small amounts of fumonisin B_4 that lacks



Fumonisin B1



AAL toxin



Sphingosine

Figure 1 Structural formulae for fumonisin B_1, AAL toxin, and sphingosine.

both hydroxyl groups at C-5 and C-10. An additional analog, fumonisin C_1, which has the same substituents as fumonisin B_1, but is 14 daltons lower in molecular weight and lacks the methyl group at C-1, has been reported (B. Branham and R. D. Plattner, submitted for publication). Fumonisin C_1 is seen in naturally contaminated corn samples and is generally present at about 1% of the amount of fumonisin B_1. Two n-acetylated fumonisins, A_1 and A_2, which were first reported by Bezuidenhout, have been reported to occur only at very low concentrations (6). Because the isolation of these components is accomplished by chromatography on silica using acetic acid in the elution solvent, they may be artifacts of isolation, as are the partially methylated fumonisins that arise during isolation from treatment of fungal culture material with acidic methanol (6). Mono-ester analogs of fumonisin B_1, B_2 and B_3, in which either C-14 or C-15 has a free hydroxyl group instead of an ester, have been isolated from cultures along with hydrolyzed fumonisins (R. D. Plattner, unpublished; 19), but these are also likely artifacts of isolation procedures. The structures reported for fumonisins are incomplete. The stereochemistry of the eight hydroxy, amino, and methyl substituents of the aminopentol backbone has not yet been reported.

Although fumonisin B_1 was identified as a new class of mycotoxin, its structure is remarkably similar to AAL toxins which are mono-esters of propane-1,2,3-tricarboxylic acid and 2,4,5,13,14-pentahydroxyheptadecane. AAL toxins are phytotoxins produced by *Alternaria alternata* (Fr.) Keissler f. sp. *lycopersici*, which causes stem canker in susceptible tomato cultivars (4, 5, 13). At the time of the discovery of the fumonisins in 1988 no animal toxicity data had been reported for AAL toxins.

Natural Occurrence of Fumonisins

Fumonisins have been detected in good quality corn in the United States and southern Africa, the only areas of the world for which extensive survey results have yet been reported. Fumonisin B_1 concentrations ranged from 0 to 38μg/g corn dry weight for 175 randomly selected whole corn samples harvested in the state of Iowa in 1988, 1989, 1990, and 1991 (32). Average concentrations of fumonisin B_1 were similar (between 2.5 and 3.5 μg/g) for the four crop years surveyed. Fumonisin concentrations in the Iowa samples were generally higher than in 38 samples of good quality home-grown corn harvested in 1985 and 1989 in the Transkei region of southern Africa (43). Average concentrations of fumonisin B_1 were 0.4 to 1.8 μg/g for the two crop years surveyed. In corn samples associated with human and animal health problems, fumonisin concentrations are generally higher than in randomly selected good quality corn. Human esophageal cancer in certain rural areas of Transkei is associated with consumption of poor quality corn that can be naturally contaminated with up to 140μg fumonisins per gram corn dry weight (43). Corn and corn-based feeds associated with field outbreaks of various animal diseases also have been well-documented to contain fumonisins, with typical ranges of 0.3–50 μg/g in Brazil (48), and 37–122 μg/g (58), 1–20 μg/g (52) and 20–330 μg/g (38) in the United States.

Little information is available on the occurrence of fumonisins in corn-based human foods. A preliminary survey of 124 samples of various products, purchased in 1990 and 1991 in the United States and southern Africa, gave a majority (74%) of samples with detectable fumonisins but only samples from the United States contained fumonisin concentrations greater than 1 μg/g (49). Ninety-eight samples of corn-based food products purchased in Switzerland in 1991 were very low in fumonisins, with all samples lower than 1 μg/g (40). Although these limited surveys indicate that consumers of corn-based foods may be ingesting fumonisins, further studies are needed to determine the prevalence of these mycotoxins.

Production of Fumonisins by Fusarium *species*

Much of the early work on production of fumonisins was done with MRC 826 of *F. moniliforme*. However, recent testing has shown that other strains of *F. moniliforme* as well as other *Fusarium* species also produce these mycotoxins. Thiel et al (51) tested *F. decemcellulare* C. Brick, *F. sporotrichioides* Sherb., *F. poae* (Peck) Wollenweb., *F. tricinctum* (Corda) Sacc., *F. avenaceum* (Fr.: Fr.) Sacc., *F. semitectum* Berk. & Ravenel, *F. camptoceras* Wollenweb. & Reinking, *F. equiseti* (Corda) Sacc., *F. acuminatum* Ellis. & Everh., *F. scirpi* Lambotte & Fautr., *F. longipes* Wollenweb. & Reinking, *F. sambucinum* Fuckel, *F. graminearum* Schwabe, *F. reticulatum* Mont., *F. compactum* (Wollenweb.) Gordon, *F. lateritium* Nees:Fr., *F. moniliforme, F. proliferatum* (T. Matsushima) Nirenberg, *F. subglutinans* (Wollenweb. & Reinking) P.E. Nelson, T.A. Toussoun, & Marasas, *F. anthophilum* (A. Braun) Wollenweb., *F. oxysporum* Schlechtend.:Fr., *F. nygamai* Burgess & Trimboli, and *F. napiforme* Marasas, P.E. Nelson & Rabie. Of the species tested, only *F. moniliforme, F. proliferatum,* and *F. nygamai* produced fumonisins.

In another study, Nelson et al (33) tested strains of *F. moniliforme* from various substrates and geographic areas for production of fumonisin B_1. They tested strains from corn-based feed from the United States, including samples from good quality corn for use in poultry rations, and from corn silks from Iowa; from millet and sorghum in Nigeria and Zimbabwe; from corn kernels from Nepal; from sorghum stalks and grain, corn kernels, sugarcane stalks, and soil from Australia; and strains isolated from patients in the United States and Canada with mycotic human keratitis and various types of cancer. They considered strains that produced < 50 μg/g of fumonisin B_1 to be low producers, those producing 50 to 500 μg/g intermediate producers, and those producing > 500 μg/g as high producers. Most of the strains from corn-based feed associated with leukoencephalomalacia were high producers (16/20), whereas the strains from millet and sorghum had fewer high producers (4/15) and a number of strains did not produce the compound. The strains from corn kernels from Nepal had only one high producer (1/10), most of the strains from good quality corn for poultry rations were high producers (7/8), and the strains from corn silks were mainly high producers (8/9). Strains from sorghum stalks and grain, corn, sugarcane, and soil from Australia were nonproducers (10/10). Several of the strains from humans (9/13) were high producers.

Two distinct mating populations, A and F, occur within the *F. moniliforme* anamorph (18, 23). Mating population A is predominant in corn while mating population F is predominant in sorghum (18). Leslie et al (23) tested 56 isolates of the teleomorph, *Gibberella fujikuroi* (Sawada) Ito in Ito & K. Kimura, that had been collected from corn and sorghum in Kansas and assigned them to mating populations based on their ability to mate with tester strains. When these isolates were grown on autoclaved corn under laboratory conditions, members of the A population produced an average of 1,786 μg/g of fumonisin B_1 while members of the F population produced an average of 7.5 μg/g. In the A mating population fumonisin B_1 production varied from 307 to 4,425 μg/g and in the F population production varied from 2 to 35 μg/g.

Nelson et al (34) tested species other than *F. moniliforme*, including *F. proliferatum*, *F. subglutinans*, *F. anthophilum*, *F. beomiforme* P. E. Nelson, T. A. Toussoun & Burgess, *F. dlamini*, Marasas, P. E. Nelson & T. A. Toussoun, *F. napiforme*, and *F. nygamai*. They found that 17/31 strains of *F. proliferatum* were high producers, 1/17 strains of *F. anthophilum* was a high producer and 2/17 strains were low producers, 5/9 strains of *F. dlamini* were low-to-intermediate producers, 5/31 strains of *F. napiforme* were low-to-intermediate producers, and 6/27 strains of *F. nygamai* were high producers. *Fusarium subglutinans* and *F. beomiforme* did not produce detectable amounts of fumonisin B_1.

In summary, survey results indicate that the potential for production of fumonisins in natural substrates and agricultural commodities certainly exists in strains from a variety of substrates and geographic areas. Of the species tested, *F. moniliforme*, *F. proliferatum*, *F. nygamai*, and *F. napiforme* are probably the most important producers of fumonisin B_1 because of their association with food grains such as corn, millet, and sorghum, and with animal mycotoxicoses such as equine leukoencephalomalacia and porcine pulmonary edema.

Animal Diseases Caused by Fumonisins

EQUINE LEUKOENCEPHALOMALACIA This neurotoxic disease of horses, donkeys, and mules is seen worldwide and is characterized by liquifactive necrosis of the white matter of one or both cerebral hemispheres (27). Equine leukoencephalomalacia has been referred to as the blind staggers, cerabritis, moldy corn disease, leukoencephalitis, corn stalk disease, encephalomyelitis, foraging disease, and cerebrospinal meningitis (57). Typical clinical signs of intoxication appear abruptly and consist of apathy, somnolent appearance with protruding tongue, reluctance to move backwards, aimless circling, and ataxia. The signs of nervous disorder become more pronounced as time passes, and the animal walks into objects apparently through lack of comprehension rather than lack of vision. Finally, the animal may become extremely excitable and frenzied, and during this period of delirium it may run wildly into objects such as fences. Death may be preceded by recumbency and paddling limb movements. The course of the disease from the onset of clinical signs to death can be extremely rapid (less than 7 hr), or it may last several days (26).

In early reports, researchers associated leukoencephalomalacia with the ingestion of moldy corn. When equidae were fed corn contaminated with *F. moniliforme*, they died of the disease. In other experiments, when corn cultures of pure strains of *F. moniliforme* were fed to horses, the animals developed leukoencephalomalacia (20, 21, 24). Horses dosed by stomach tube with culture material of *F. moniliforme* MRC 826 grown on corn developed severe hepatosis and mild edema of the brain. A horse injected with purified fumonisin B_1 seven times during the first 9 days of an experiment developed clinical signs on day 8 that included nervousness followed by apathy, a wide-based stance, trembling, ataxia, reluctance to move, paresis of the lower lip and tongue, and an inability to eat or drink. The horse was euthanized on the 10th day and the principal lesions were severe edema of the brain and early, bilaterally symmetrical, focal necrosis in the medulla oblongata (24). In a later experiment, leukoencephalomalacia was induced by the oral administration of fumonisin B_1. Two horses were dosed and developed nervous signs such as apathy, changes in temperament, lack of coordination, walking into objects, and paralysis of the lips and tongue. Characteristic lesions of leukoencephalomalacia were present in the brains of both horses (17). These two experiments (17, 24) prove conclusively that fumonisin B_1 can induce leukoencephalomalacia in horses.

PORCINE PULMONARY EDEMA Pulmonary edema in swine caused by feeding *F. moniliforme* propagated on corn in bulk was reported by Kriek et al (20). Two pigs were dosed by feeding bulk culture material in the ration and both developed pulmonary edema. The next report on porcine pulmonary edema was in 1990 (15) when simultaneous epidemics were observed on two southwest Georgia farms, resulting in the death of 34 mature swine. Gross pathological changes observed included extremely marked pulmonary edema and massive hydrothorax. The thoracic cavities were overfilled with golden-yellow liquid. Routine diagnostic testing for toxins and infectious agents failed to establish an etiology and these epidemics appeared to represent an unrecognized disease (15).

Feed consisting of corn screenings from the 1989 crop was common to both farms where this problem occurred. Death began about 5 days after the screenings were first fed and ceased 24 hr after screenings were removed as the feed source. On day 7 of the feeding study, one animal was found dead and a severely dyspneic animal was euthanized the same day. At necropsy both animals exhibited marked pulmonary edema and hydrothorax previously seen in field cases. Corn screenings were cultured and *F. moniliforme* was recovered from feed samples from both farms. Preliminary data showed that the concentration of fumonisin B_1 ranged from 105 μg/g to 155 μg/g in the two feed samples (15). Additional tests were done on pigs with pure fumonisin B_1 and fumonisin B_2. One pig was injected daily with pure fumonisin B_1 and died on day 5. This pig developed pulmonary edema and exhibited lesions similar to those observed in the field and in other experimental cases (15).

During the 1989 corn harvest season, numerous outbreaks of porcine pulmonary edema were reported and were generally confined to the central United States (44). In almost all cases, feed containing corn and/or corn screenings from the 1989 harvest was implicated as the causal factor. Because a porcine pulmonary edema-like syndrome caused by feeding culture material of *F. moniliforme* to swine was reported earlier (20), feed samples were collected for mycological evaluation and chemical analyses. Five feed samples associated with porcine pulmonary edema cases were obtained from farms in southeastern Iowa and were comprised primarily of corn and/or corn screenings. *Fusarium moniliforme* was isolated from all samples and *F. proliferatum* was isolated from one sample. The isolates of *F. moniliforme* from feed produced fumonisin B_1 in amounts ranging from 900 to 2350 μg/g and fumonisin B_2 in amounts ranging from 120 to 350 μg/g when grown in corn cultures. The single isolate of *F. proliferatum* produced 1670 μg/g of fumonisin B_1 and 150 μg/g of fumonisin B_2 when grown in corn culture. These data (44) and those of Harrison et al (15) indicate that fumonisin B_1 is probably the cause of porcine pulmonary edema.

Ross et al (44) isolated both *F. moniliforme* and *F. proliferatum* from feed samples that were not associated with any on-farm problems such as porcine pulmonary edema. The isolates of *F. moniliforme* from nonproblem feeds produced fumonisin B_1 in amounts ranging from 1310 to 1590 μg/g when grown in corn culture. An isolate of *F. proliferatum* from the same source produced 2790 μg/g of fumonisin B_1 when grown in corn culture. The production of high concentrations of fumonisins by isolates from both problem and nonproblem feeds suggests the potential for fumonisin contamination in any feed containing *F. moniliforme* and/or *F. proliferatum*.

EXPERIMENTAL LIVER CANCER Kriek et al (21) isolated 21 strains of *F. moniliforme* (*F. verticillioides*) from corn crops in South Africa and the Transkei. These strains did not produce moniliformin, but the majority were toxic to ducklings. Acute mortality was common in ducklings fed fungal-corn culture material of these isolates. In rats the mean time to death was at least 24 days. Cirrhosis and nodular hyperplasia of the liver, and the occurrence of acute and proliferative endocardial lesions and concurrent intraventricular thrombosis were encountered frequently (20). In a later study (25), culture material on corn of strain MRC 826 of *F. moniliforme* was fed to rats on a lifelong basis. At a dietary concentration of 8%, culture material was hepatotoxic and caused 100% mortality. Hepatic lesions in rats that died were characterized by cirrhosis, nodular hyperplasia, and bile-duct proliferation. At lower dietary concentrations, culture material was hepatocarcinogenic and caused hepatocellular carcinoma and ductular carcinoma of the liver. No hepatocellular or ductular carcinomas occurred in the controls. Hepatocellular carcinomas in the experimental rats invariably developed in severely cirrhotic livers showing nodular hyperplasia. Adenofibrosis also developed concurrently with hepatocellular carcinoma (25).

In another experiment (36, 56), corn that was being used as feed during an epidemic of leukoencephalomalacia was obtained from a farm in which 9 of 15 horses died. This corn, in which there was no history of fungicide treatment, was ground finely and fed unsupplemented to rats. *F. moniliforme* was the predominant fungal species recovered from this corn. Analysis of the feed for aflatoxins at the 0.9 μg/g level gave negative results. Control rats euthanized and evaluated on day 176 were free of significant gross lesions. Gross lesions in all test rats necropsied from 123 to 176 days post-feeding were confined to the liver and consisted of multiple hepatic nodules and pale depressed hepatic areas. Histological examination revealed multiple hepatic neoplastic nodules and large areas of adenofibrosis and cholangiocarcinomas. This study was the first report of the hepatocarcinogenicity of a sample of equine feed infested with *F. moniliforme* (36, 56).

Gelderblom et al (10) fed a corn-based diet containing 50 mg/kg of partially pure (not less than 90%) fumonisin B_1, isolated from culture material of strain MRC 826 of *F. moniliforme*, to a group of 25 rats over a period of 26 months. A control group of 25 rats received the same diet without fumonisin B_1. The liver was the main target organ in fumonisin B_1-treated rats, and the hepatic pathological changes were identical to those previously reported in rats fed culture material of *F. moniliforme* MRC 826. All fumonisin B_1-treated rats that died or were killed from 18 months onward suffered from a micro- and macronodular cirrhosis, and they had large expansile nodules of cholangiofibrosis at the hilus of the liver. Ten of 15 fumonisin B_1-treated rats (66%) that were killed and/or died between 18 and 26 months developed primary hepatocellular carcinoma. Metastases to the heart, lungs, or kidneys were present in four of the rats with hepatocellular carcinoma. No neoplastic changes were observed in the esophagus, heart, or forestomach of fumonisin B_1-treated rats, and this is contrary to previous findings when culture material of the fungus was fed to rats. It was concluded that fumonisin B_1 is responsible for the hepatocarcinogenic and the hepatotoxic but not all of the other toxic effects of culture material of *F. moniliforme* MRC 826 in rats (10).

MODE OF ACTION The diverse toxicological effects of fumonisins on a variety of animals have led to extensive research on their biochemical mode of action. The equine neurotoxicity of fumonisins and their structural similarity to the sphingolipid precursor, sphingosine (Figure 1), led Wang et al (54) to propose that fumonisins affect sphingolipid metabolism. In primary rat hepatocytes and in pig kidney epithelial cell cultures, fumonisin B_1 inhibited (EC_{50} = 0.1 μM) the biosynthesis of sphingosine and increased the level of sphinganine, a sphingosine precursor. Further studies with rat liver microsomes suggested that fumonisin specifically inhibits the enzyme sphingosine N-acetyl transferase (37, 54). Although sphingolipids are particularly rich in neuronal tissues, they are also important membrane components of many other types of eukaryotic cells, and they appear to be involved in the regulation of cell growth and differentiation (31). Thus, fumonisin inhibition of sphingolipid metabolism could be at the core of a wide range of physiological effects, including tumor promotion.

Significance of Fumonisins in Plant Disease

The high frequency of fumonisin contamination in corn and of fumonisin production among strains of *F. moniliforme* isolated from corn raises the possibility that fumonisins are pathogenicity or virulence factors in disease on corn. Further indirect evidence of a role for fumonisins in plant disease is provided by their structural similarity to AAL-toxin (Figure 1), which is a pathogenicity factor of *Alternaria alternata* f. sp. *lycopersici* in stem canker of tomato (13). Very little is known about the phytotoxicity of fumonisins, but recent experiments indicate that they are toxic to some plant tissues. Gilchrist and co-workers (14) showed that 0.4 μM fumonisin B_1 caused necrosis on detached leaflets of an *Alternaria*-susceptible tomato cultivar; AAL-toxin had a 20-fold higher specific activity in the same leaflet bioassay. Furthermore, fumonisin B_1 was equal to AAL-toxin (20 μM threshold) in causing necrosis on detached leaflets of a resistant tomato cultivar (14). A preliminary study found no effect of fumonisin B_1 solutions on growth of intact corn seedlings, but did not address whether fumonisins were actually taken up by seedlings (1).

Genetic analysis is a powerful technique for critical analysis of the role of fumonisins in *Fusarium* diseases of corn and other plants. *Fusarium moniliforme* mating population A has many advantages for the genetic analysis of fumonisin biosynthesis and for evaluating the significance of fumonisin in plant disease. *Gibberella fujikuroi*, the teleomorph of this species, is a heterothallic ascomycete that can complete its life cycle in a few weeks under laboratory conditions and that can be transformed using standard techniques of molecular biology. Although fumonisin-producing strains predominate in mating population A, a few nonproducing strains have been identified in this mating population, and genetic analyses of fumonisin biosynthesis and virulence are under way. Heritability of fumonisin production was studied by crossing fumonisin-producing strains with nonproducing strains. In the three crosses analyzed, the ability to produce fumonisins segregated as a single gene or group of closely linked genes (8). Virulence of both parents and of more than forty random ascospore progeny of one cross was assessed by their ability to cause root and stem rot of corn seedlings grown in soil infested with the respective ascospore isolates. High virulence segregated with high fumonisin production; all fumonisin nonproducing progeny were low in virulence on corn seedlings (A. F. Desjardins, R. D. Plattner & J. F. Leslie, unpublished). These results suggest that either fumonisin production is required for high virulence on corn or that a gene for fumonisin biosynthesis is closely linked to a gene or genes for virulence.

Selected Methods for Production and Analysis of Fumonisins

To provide a quick reference for those interested in producing fumonisins for biological studies, we present a selection of methods for screening isolates for the ability to produce fumonisins in liquid media and on solid substrates, for isolation and purification of fumonisins, and for chemical analysis. It must be stressed that culture materials of *F. moniliforme* and fumonisins are potential health hazards. Therefore, proper guidelines for safe handling of these mycotoxins in the laboratory should be followed.

PRODUCTION OF FUMONISINS ON CORN KERNELS Fumonisins can be readily produced by strains of *F. moniliforme*, *F. proliferatum*, and related species on autoclaved corn kernels (35). For large-scale production, 500 g of yellow corn kernels and 500 ml of distilled water are added to a 30.5 × 61 cm autoclavable polyethylene bag. The corn is inoculated by drawing an aqueous suspension from a lyophilized culture into a sterile 5 ml syringe fitted with a 19-gauge needle and injecting 1 ml through the side of each bag. Bags of inoculated corn are incubated in the dark at 20 to 22°C for four weeks. Seven to eight days after inoculation, holes are punched near the tops of the bags to promote aeration. After a four-week incubation period, cultures are soaked overnight in chloroform/acetone (50/50, V/V) in 4-liter flasks to kill the fungus. This process is followed by filtering through 2 mm nylon mesh screens and air drying the culture material for 24 to 48 hr. The three most important factors in the production of fumonisins in bulk corn cultures are temperature control, moisture, and aeration. By this method yields of 2–4 g fumonisins per kg of culture material can be routinely obtained from isolates capable of producing large amounts of fumonisins.

When screening large numbers of isolates for their ability to produce fumonisins, coarsely cracked corn (50 g + 11 ml water) in 300 ml erlenmeyer flasks with Morton closures is autoclaved for 30 min. After autoclaving, an additional 11 ml of sterile distilled water is added aseptically to each flask. Conidial suspensions for inoculation of flasks are prepared by washing 7–14 day-old cultures on agar slants with sterile distilled water. Each flask is inoculated with 10^7 conidia and shaken once or twice daily for three days to distribute the inoculum. The flasks are incubated in the dark at 25°C ± 2°C for four weeks. Uniform distribution of the inoculum appears to be necessary to insure that fumonisins are produced. Clumps of corn in cultures can result in anaerobic growth of the fungus and lead to drastically lower concentrations of fumonisin B_1 in the culture.

PRODUCTION OF FUMONISINS IN LIQUID MEDIA Production of fumonisins in static liquid cultures of *F. moniliforme* has been reported by Jackson & Bennett (16) and Plattner & Shackelford (41). In these studies, concentrations of fumonisins were rather low (20–50 mg/L) and required relatively long incubation periods (14–25 days). Methods for rapid production in liquid culture are still under development in several laboratories. Recently, larger amounts of fumonisins have been obtained under shake conditions using the following medium and procedures modified from the procedures used to produce AAL toxin (13). The medium is prepared from three sterilized solutions. First, six items (8.0 mM L-asparagine, 1.7 mM NaCl, 4.4 mM K_2HPO_4, 2.0 mM $MgSO_4$, 8.8 mM $CaCl_2$, and 0.05% yeast extract) are prepared (at 2× final concentration), adjusted to pH 3.0 with H_3PO_4 and autoclaved. Glucose (0.24 M) is autoclaved separately (5× concentration). Finally, malic acid (5.0 mM) is filter- sterilized at 10× concentration and added. Spore suspensions of 7-day-old cultures of *F. moniliforme* are used to inoculate the medium at 5×10^3 spores/ml of culture media in erlenmyer flasks with Morton closures. Flasks are shaken in the dark at 200–220 RPM at a temperature of 25–28° C. Production of fumonisin B_1 begins around day 4 and reaches maximum levels of 200–300 μg/ml by day 21. Yields of fumonisin B_1 production of 500–600 μg/ml were achieved by modifying C/N ratios to 48:1 and carbon content to 18–36 μg/μl (B. Branham, unpublished).

EXTRACTION OF FUMONISINS FROM CULTURES In liquid cultures, the fumonisins are found in the culture filtrate and extraction is not necessary. Extraction of fumonisins from solid cultures is achieved with either methanol/water (3/1), acetonitrile/water (1/1), or water. Acetonitrile/water extraction (5 ml/g culture material) produces the highest yields of fumonisins. Water is considerably less efficient at extracting fumonisins B_2 and B_3, and larger ratios of water to sample (>20 ml/g) are required to completely extract fumonisin B_1. Fumonisins are stable in water or in acetonitrile/water mixtures. Some methylation occurs when fumonisin is kept in methanol, and partial hydrolysis of fumonisin can occur in acidic solvents over extended periods of time.

METHODS OF ANALYSIS FOR FUMONISINS Purification of fumonisins can be unexpectedly difficult and time consuming. Fumonisins contain a free primary amine and multiple free carboxyl groups that can carry positive and negative charges. Subtle changes in pH or ionic strength in the complex extracts from which they are purified can sometimes unexpectedly affect chromatographic behavior. The original report of fumonisin B_1 by the research group in South Africa (9) describes methods for isolation and purification. A second excellent paper by that group (6) elaborates and expands on the separation of the other fumonisins. In our laboratory, we initially separated fumonisins from the aqueous acetonitrile extracts of solid cultures, or from liquid culture filtrates, by HPLC on 37–55 μm 125 A Bondapak C18 (47 × 300 mm) PrePak cartridges (WATERS) using a water-to-methanol gradient. In this chromatography, unlike the reported XAD-2 method (6), separation between fumonisin B_1, B_2, and B_3 is achieved. Recoveries of all three major fumonisins from this step are good (80–90%). The subsequent purification of the fumonisin B_1-containing fraction is relatively straightforward. This fraction is chromatographed twice on a 8-μm 60 A Dynamax C18 (41.4 × 250 mm) column (Ranin) using eluting solvents of methanol/water/acetic acid (65/35/a1) and then acetonitrile/water/acetic acid (70/30/1).

Highly purified fumonisins are colorless liquids. Pigments are carried along with the separation procedure and removing the last traces of these yellow and brown pigments has proven difficult. These pigments are much less abundant in liquid culture filtrates than in corn extracts. Reference standards of fumonisins B_1 and B_2 are available commercially.

Fumonisin can easily be detected at microgram levels by Thin Layer Chromatography (TLC) on C18 reverse phase or on normal phase silica plates. Methanol/water (3/1) is a suitable development solvent for C18 plates. On normal phase silica plates, several development systems are suitable. In plates developed with chloroform/methanol/acetic acid (6:3:1) or acetonitrile/water (85:15), fumonisin B_1 moves about 1/4 the distance of the solvent (rf 0.25). The fumonisins appear as light to dark purple spots after spraying the plate with p-anisaldehyde (9), followed by heating. Alternately, fumonisins can be visualized under shortwave ultraviolet (UV) light as fluorescent spots after spraying the developed TLC plate with a solution of fluorescamine (45). Because normal phase plates are more economical, they are recommended over reverse phase plates. The detection limit for TLC with p-anisaldehyde is about 100 ng on the plate, but when crude extracts are spotted, interfering components that co-migrate with the fumonisins can obscure detection.

It is our experience that, without prior clean-up, fumonisins cannot be reliably detected in extracts of culture filtrates at concentrations below about 100–200 μg/g. Despite this limitation, TLC can still be useful for screening of crude culture extracts because fumonisin-producing strains often produce well in excess of 1000 μg/g. Lower detection limits require sample clean-up using either the methods presented by Sydenham (50) or those of Ross (44).

Fumonisins are saturated long chain compounds that have no UV adsorbing chromophore, nor do they fluoresce. Therefore, they are difficult to detect by high performance liquid chromatography (HPLC) without derivatization. Accordingly, several methods have been developed that rely on derivitization of the free primary amine group with UV-absorbing or fluorescent derivatives. One simple method involves derivatization of the sample with o-phthaldialdehyde (OPA) reagent to form a fluorescent derivative (50). This derivative is not stable for more than a few minutes and must be analyzed quickly after formation for reproducible results. Culture filtrates of liquid cultures can be analyzed at concentrations as low as 1 mg/L without any sample clean-up. Twenty-five microliters of culture filtrate is reacted with 100 μl of OPA reagent (40 mg OPA + 5 ml 0. 1 M disodium tetraborate + 50 μl 2-mercaptoethanol) and 10 μl are injected into the HPLC after 1 min of reaction time. The LC column is a 3 cm × 4/6 mm 3 μ Pecosphere C18 column. The solvent is methanol/0.1 M sodium dihydrogen phosphate (70 = 30 adjusted to pH 3.35 with o-phosphoric acid) at 1 ml/min. Fumonisins are detected by fluorescence with excitation at 335 nm and emission at 440. Extracts of solid cultures can also be measured without prior clean-up at fumonisin B_1 concentrations as low as about 25 μg/g. At lower concentrations, which are found in naturally contaminated samples, sample clean-up prior to the analysis by HPLC is necessary for reliable results. Sample clean-up on strong anion exchange (SAX) columns (50) works well, but care must be taken with this procedure and control, and spiked blank control samples must be run to verify that recovery of the clean-up procedure is adequate.

SUMMARY

Fusarium moniliforme is one of the most prevalent fungi associated with corn, and since the early 1900s it has been suspected of being involved in animal and human diseases. Moldy corn infested with *F. moniliforme* has been associated with field outbreaks of a disease in horses called leukoencephalomalacia and more recently with a disease in swine called pulmonary edema. In addition, corn infested with this fungus has been associated with high rates

of human esophageal cancer in southern Africa. When selected strains of this fungus were grown on corn and fed to rats, the material caused liver cancer. Until 1988, the toxin or toxins responsible for these diseases were unknown. In 1988, the fumonisins, a new class of mycotoxins, were characterized and purified from corn cultures of *F. moniliforme* and shown to cause leukoencephalomalacia in horses, pulmonary edema in swine, and to be a liver carcinogen in rats. Although the initial work on fumonisins was done with material produced by *F. moniliforme,* subsequent studies have shown that other *Fusarium* species such as strains of *F. proliferatum* and *F. nygamai* may also produce large amounts of fumonisins.

Fumonisins have been detected in good quality corn produced in several areas in the United States. Corn and corn-based feeds associated with field outbreaks of various animal diseases in the United States have been well-documented to contain fumonisins in concentrations of 1–330 μg/g. Little information is available on the occurrence of fumonisins in corn-based human foods. A preliminary survey of 124 samples of various products, purchased in 1990 and 1991, showed that 74% contained detectable fumonisins and some samples had concentrations greater than 1 μg/g.

Fumonisins pose a potential health threat to both animals and humans, and these compounds cause several animal diseases. Although limited surveys indicate that human consumers of corn-based foods may be ingesting fumonisins, further studies are needed to determine both the prevalence and tolerance levels of these mycotoxins in foods produced for human consumption.

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